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(54) BACTERIOCHLOROPHYLL-A DERIVATIVES USEFUL IN PHOTODYNAMIC THERAPY

BAKTERIOCHLOROPHYLL-A-DERIVATE BEI FOTODYNAMISCHER THERAPIE

DERIVES DE BACTERIOCHLOROPHYLLE-A UTILES A LA THERAPIE PHOTO-DYNAMIQUE

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Description

The invention relates to the field of photodynamic therapy and related treatment of in vitro samples using light-absorbing resonant ring systems and irradiation. More specifically, the invention is directed to methods relating to in vivo photodynamic therapy and diagnosis and in vitro sterilization using bacteriochlorophyll-a and its related compounds.

Photodynamic therapy using porphyrins and related compounds has, by now, a fairly long history. Early work, in the 1940s, demonstrated that porphyrin could be caused to fluoresce in irradiated tumor tissue. The porphyrins appeared to accumulate in these tissues, and were capable of absorbing light in situ, providing a means to detect the tumor by the location of the fluorescence. A widely used preparation in the early stages of photodynamic treatment both for detection and for therapy was a crude derivative of hematoporphyrin, also called hematoporphyrin derivative, HpD, or Lipson derivative prepared as described by Lipson and coworkers in J Natl Cancer Inst (1961) 26:1-8. Considerable work has been done using this preparation, and Dougherty and coworkers reported the use of this derivative in treatment of malignancy (Cancer Res (1978) 38:2628-2635; J Natl Cancer Inst (1979) 62:231-237).

Dougherty and coworkers prepared a more effective form of the hematoporphyrin derivative which comprises a portion of HpD having an aggregate weight >10 kd. This form of the drug useful in photodynamic therapy is the subject of U.S. Patent 4,649,151, is commercially available, and is in clinical trials.

The general principles of the use of light-absorbing compounds, especially those related to porphyrins, has been well established as a treatment for tumors when administered systemically. The differential ability of these preparations to destroy tumor, as opposed to normal tissue, is due to the homing effect of these preparations to the objectionable cells. (See, for example, Dougherty, T.J., et al., "Cancer: Principles and Practice of Oncology" (1982), V.T. de Vita, Jr., et al., eds., pp. 1836-1844.) Efforts have been made to improve the homing ability by conjugating hematoporphyrin derivative to antibodies. (See, for example, Mew, D., et al., J Immunol (1983) 130:1473-1477.) The mechanism of these drugs in killing cells seems to involve the formation of singlet oxygen upon irradiation (Weishaupt, K.R., et al., Cancer Research (1976) pp. 2326-2329).

The use of hematoporphyrin derivative or its active components in the treatment of skin diseases using topical administration has also been described in U.S. Patent 4,753,958. In addition, the drugs have been used to sterilize biological samples containing infectious organisms such as bacteria and virus (Matthews, J.L., et al., Transfusion (1988) __ :81-83). Various other photosensitizing compounds have also been used for this purpose, as set forth, for example, in U.S. Patent 4,727,027.

In general, the methods to use radiation sensitizers of a variety of structures to selectively impair the functioning of biological substrates both in vivo and in vitro are understood in the art. The compounds useful in these procedures must have a differential affinity for the target biological substrate to be impaired or destroyed and must be capable of absorbing light so that the irradiated drug becomes activated in a manner so as to have a deleterious effect on the adjacent compositions and materials.

Because it is always desirable to optimize the performance of therapeutics and diagnostics, variations on the porphyrin drugs traditionally used in treatment and diagnosis have been sought. A number of general classes of photosensitizers have been suggested including phthalocyanines, psoralen-related compounds, and multicyclic compounds with resonant systems in general. Most similar to the compounds disclosed herein are various pheophorbide derivatives whose use in photodynamic therapy has been described in EPO Application 220686 to Nihon Metaphysics Company; ethylene diamine derivatives of pheophorbide for this purpose described in Japanese Application J85/000981 to Tama Seikayaku, K.K., and Japanese Application J88/004805 which is directed to 10-hydroxy pheophorbide-a. In addition, pheophorbide derivatized to a long chain hydrocarbyl group has been disclosed as useful in photodynamic therapy in U.S. Serial No. 221,804, filed 20 July 1988, assigned to the same assignee and incorporated herein by reference. In addition, Beems, E.M., et al., in Photochemistry and Photobiology (1987) 46:639-643 discloses the use as photosensitizers of two derivatives of bacteriochlorophyll-a -- bacteriochlorophyllin-a (also known as bacteriopheophorbide-a, which lacks the phytyl alcohol derivatized in bacteriochlorophyll-a) and bacteriochlorin-a (which lacks both the phytyl group and the Mg ion). These authors direct their attention to these derivatives as being advantages on the grounds of enhanced water solubility as compared to bacteriochloro-phyll-a.

The problem remains to find suitable photosensitizers useful in photodynamic therapy and diagnosis which are optimal for particular targets and particular contexts. It is unlikely whether a single compound or small group of compounds, while generally applicable, would be of maximum benefit in every instance. Thus, the invention provides an additional group of photosensitizing compounds which becomes part of the repertoire of candidates for use in specific therapeutic and diagnostic situations.

Lasers in surgery and medicine, supplement 1, abstract no. 148, page 36, 15th-17th April 1989 disclose the application of diode lasers to photodynamic therapy. It indicates that a search had begun for appropriate sensitizers and that three classes of such materials had been identified: tetrahydroporphyrins such as bacteriochlorophyll, napthocyanines such as silicon-napthocyanine and a 5-nitrogen porphyrin-like ring systems such as texaphyrin.

Proceedings, SPIE - The International Society for Optical Engineering, 1065, 2-10, January 1989 compares various different photosensitizers to Photofrin II (trade mark) with respect to their potential for causing direct tumor cell inac-

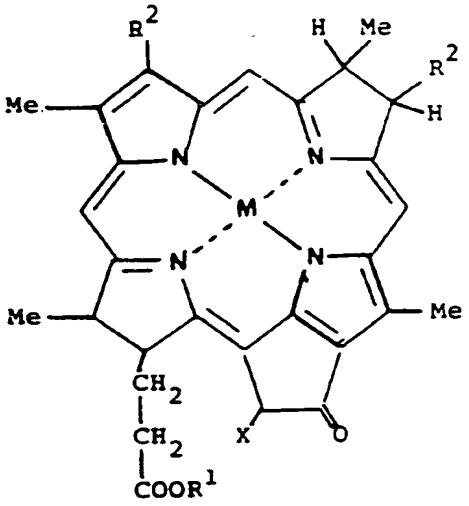
tivation and/or vascular damage. Bacteriochlorophyll was one compound tested. In one experiment bacteriochlophyll was administered to mice in an amount of 35 mg/kg body weight, tumors from the mice were excised and the tumors then subjected to light of wavelength 780 nm.

Journal of photochemistry and photobiology, 1, 93-101, 1987 gives a photophysical study of bacteriochlorophyll a and bacteriopheophytin-a singlet oxygen generation.

The invention provides alternative methods of photodynamic therapy and diagnosis using a group of compounds related to the tetrahydroporphyrins, such as bacteriochlorophyll-a or -b or the corresponding bacteriochlorins.

The present invention provides an ex vivo method to effect the destruction or impairment of undesired target biological substrates in a biological fluid which method comprises:

a) treating said biological substrates with a compound of formula (1) or (2):



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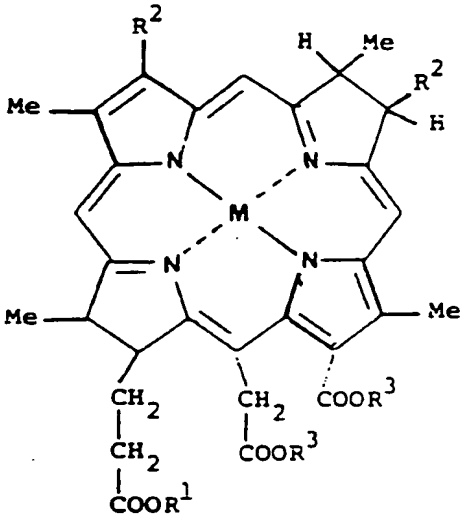


(2)

wherein M is a non-paramagnetic metal selected from Mg^{2+} Sn^{2+}, and Zn^{2+}, or represents 2 H^+, each H^+ bonded to one of the N atoms connected by the solid lines;

R^1 is a saturated or unsaturated hydrocarbyl residue of 8-25 carbon atoms;

each R^2 is independently selected from vinyl, ethyl, acetyl and 1-hydroxyethyl; and

X is $COOR^3$, wherein R^3 is C_{1-4} alkyl;

in an amount of 1 to 100 µg/ml to photosensitize said biological substrates to the resultant of irradiation absorbed by the compound of formula (1) or (2); and

(b) irradiating the treated biological substrates with radiation having a wavelength absorbed by the compound of formula (1) or (2).

The present invention further provides the use of a compound of formula (1) or (2) in the manufacture of a composition for use in a method to effect the destruction or impairment of an undesired biological substrate or to locate a tumor in a subject, which method comprises:

administering said composition to said subject in an amount of 1 to 20 mg/kg body weight of the compound of formula (1) or (2); and
irradiating said undesired biological substrate or said tumor in vivo with radiation having a wavelength absorbed by the compound of formula (1) or (2).

The present invention also provides the use of a compound of formula (1) or (2) in the manufacture of a composition for use in a method to effect the destruction or impairment of a pathogen, which comprises:

administering said composition to a subject infected with said pathogen in an amount of 1 to 20 mg/kg body weight of the compound of formula (1) or (2); and
irradiating tissue or fluid containing said pathogen in vivo or in vitro with radiation having a wavelength absorbed by the compound of formula (1) or (2).

The present invention additionally provides the use of a compound of formula (1) or (2) in the manufacture of a composition for use in a method of treatment of a skin disease, which comprises:

topically applying said composition to a subject with said skin disease in an amount of 1 to 20 mg/kg body weight of the compound of formula (1) or (2); and
irradiating the infectious virus or cells carrying the disease with radiation having a wavelength absorbed by the compound of formula (1) or (2).

The present invention yet further provides a composition for use in a method to effect the destruction or impairment of undesired target biological substrates by photodynamic treatment which comprises a compound of formula (1) or (2) in admixture with at least one pharmaceutically acceptable excipient which contains a liposome carrier.

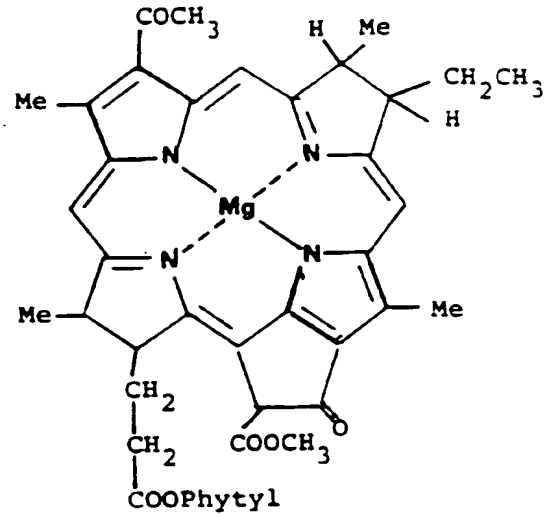
Thus, in one aspect, the invention concerns a method to effect the impairment or destruction of a target biological substrate which method comprises treating the target substrate with an amount of the compound of formula (1) effective to photosensitize said substrate followed by irradiating said target substrate with radiation in a wavelength band absorbed by the compound of formula (1) for a time effective to impair or destroy the substrate.

Figure 1 is a table showing the results of treatment with bacteriochlorophyll-a at a fixed total radiation energy.

Figure 2 shows the action spectrum constructed from the table of Figure 1.

Figure 3 shows the tumor response as compared to foot sensitization to bacteriochlorophyll-a as a function of time.

Bacteriochlorophyll-a (bchla) is a tetrahydroporphyrin found in certain photosynthetic bacteria, for example, Rhodopseudomonas viridis. Bchla has the formula:



Bchla is essentially identical to the chlorophyll-a of higher plants except that ring B is in the dihydro form and the vinyl group in ring A is converted to an acetyl group. The wavelength absorption maximum of bchla is about 780 nm and the extinction coefficient in this region is quite high (E_{780} = 75,000). This long wavelength absorption is advantageous because light penetrates tissues 2-3 times more effectively at a wavelength of nearly 800 nm versus lower wavelengths, e.g., 630 nm.

Bchla is readily obtained by extraction from bacterial sources, and is available commercially from Porphyrin Products, Logan, UT. Although the material is readily oxidized, especially in the presence of light, and the magnesium ion is readily removed in the presence of dilute acid, bchla is sufficiently stable in vivo to be an effective phototherapeutic agent.

In bacteriochlorophyll-b, which can also readily be obtained from bacterial sources, R^2 in the B ring is vinyl rather than ethyl. The other embodiments of R^2 can easily be prepared starting with bacteriochlorophyll-b by standard hydration of the vinyl group to obtain the 1-hydroxyethyl substituent, and mild oxidation to obtain the corresponding acetyl substituent. Similarly, the R^2 substituent in ring A can be reduced to the 1-hydroxyethyl and/or dehydrated to vinyl and/or reduced to ethyl.

Conversion of the compounds of formula (1) to the compounds of formula (2) can readily be effected by opening of the cyclopentanone ring using known reagents, such as alkaline solution in the presence of oxygen as described in "Porphyrins and Metalloporphyrins", Smith, K., ed. (1975) Elsevier Press, pp. 52-53. Although the phytyl group is removed in this reaction, reesterification to the desired R^1 can be effected by standard methods.

In general, alternate embodiments of R^1 or R^3 in either formula (1) or formula (2) can be obtained by transesterification or by hydrolysis and reesterification. In some instances, this esterification should be conducted on the compounds when they are in the form of the corresponding porphyrin or dihydroporhryrins obtained by oxidation, for example, using osmium tetroxide and then re-reducing to the tetrahydro form. In all of the conversions set forth above, it may be necessary to conduct the reactions in a certain order, to restore or remove the metal substituent and/or to utilize protective reactions and groups as is understood by practitioners in the art.

The compounds of formulae (1) and (2) are used for photodynamic therapy and diagnosis with respect to target biological substrates. By "target biological substrate" is meant any cells, viruses or tissues which are undesirable in the environment to which therapy or other corrective action, such as sterilization, is employed, or the location of which is desired to be known in an environment to which diagnosis is applied. For example, in a manner analogous to the use of the active fraction of hematoporphyrin derivative (Hpd), as described in U.S. Patent 4,649,151, incorporated herein by reference, neoplastic tissue is effectively treated in vivo by virtue of the ability of the drug to accumulate preferentially in such tissue, and by virtue of the photosensitizing nature of the drug. In this instance, the target biological substrate is the neoplastic tissue. As described in this patent, the drug is injected into the subject, and permitted to clear normal tissue. Then the neoplastic tissue is exposed to radiation at a wavelength appropriate to its absorption spectrum. The patent further describes the synergistic effect of heat supplied, if desired, by infra-red irradiation. In addition, the location of the tumor can be ascertained by the fluorescence of the drug.

In another application, Matthews, J.L., et al., Transfusion (1988) __:81-83, describe the use of the photosensitizing compounds HpD and the active fraction thereof, designated DHE, in eradicating pathogens from fluids in vitro. This article describes techniques for treating blood or other biological fluids to eliminate pathogens such as protozoa, virus, bacteria, fungi, and so forth. Similarly, U.S. Patent 4,727,027 describes the use f furocoumarin in conjunction with irradiation by UV light for decontamination of blood products. In these instances, the target substrates are pathogens which may include a variety of "organisms" such as viruses and protozoa, as well as bacteria and fungi.

In U.S. Patent 4,753,958, topical treatment of skin diseases using photosensitizing drugs is described. In this instance, the target biological substrate is the infectious virus or cell carrying the disease. This too, may be a virus, bacterium, or other microorganism, including fungal infections.

For use in the method of the invention, the compounds of formulae (1) and (2) are formulated using conventional excipients appropriate for the intended use. For systemic administration, in general, buffered aqueous compositions are employed, with sufficient nontoxic detergent to solubilize the active compound. As the compounds of formulae (1) and (2) are generally not very soluble in water, a solubilizing amount of such detergent is employed. Suitable nontoxic detergents include Tween-80, various bile salts, such as sodium glycholate, various bile salt analogs such as the fusidates. Alternate compositions utilize liposome carriers. The solution is buffered at neutral pH using conventional buffers such as Hank's solution, Ringer's solution, or phosphate buffer. Other components which do not interfere with the activity of the drug may also be included, such as stabilizing amounts of proteins, for example, serum albumin.

Systemic formulations can be administered by injection, such as intravenous, intraperitoneal, intramuscular, or subcutaneous injection, or can be administered by transmembrane or transdermal techniques. Formulations appropriate for transdermal or transmembrane administration include sprays and suppositories containing penetrants, which can often be the detergents described above.

For topical local administration, the formulation may also contain a penetrant and is in the form of an ointment, salve, liniment, cream, or oil. Suitable formulations for both systemic and localized topical administration are found in Remington's Pharmaceutical Sciences, latest edition, Mack Publishing Co., Easton, PA.

For use ex vivo to treat, for example, blood or plasma for transfusion or preparations of blood products such as Factor VIII, no special formulation is necessary, but the compounds of formula 1 and 2 are dissolved in a suitable compatible solvent and mixed into the biological fluid at a concentration of 1-100 µg/ml prior to irradiation.

For photodynamic therapeutic and diagnostic applications, suitable dosage ranges will vary with the mode of application and the choice of the compound, as well as the nature of the condition being treated or diagnosed. Preferred suitable dosages are 1 to 3 mg/kg bodyweight. For topical administration, typically amounts of the order of 50-100 mg total are employed.

The general procedures for photodynamic therapy and diagnosis in vivo are analogous to those described in U.S. Patent 4,649,141; those for ex vivo treatment are analogous to those described by Matthews, J.L., et al., Transfusion (supra); topical methods are analogous to those described in U.S. Patent 4,753,958; all are incorporated herein by reference.

Briefly, for systemic administration, a suitable time period after administration, typically from several hours to two days is allowed to elapse in order to permit concentration of the drug of formula (1) or (2) in the target biological substrate. In general, this substrate will be a tumor, and the localization of the compound of formula (1) or (2) can be monitored by measuring the fluorescence or absorption of the target tissue as compared to background. After localization has been accomplished, the target biological substrate is irradiated with a suitable band of irradiation, in the case of the compounds of formula (1), in the range of 750-800 nm at a rate of 5 mW/cm^2 -0.75 W/cm^2, and a total energy of 100-1000 J/cm^2.

For topical treatment, localization is immediate, and the corresponding radiation can be provided immediately. For treatment of biological fluids ex vivo, again, no localization interval is required, and radiation is applied on the order of 1-10 J/cm^2. Because penetration of tissue is not required, lower total energy can be employed.

The following Examples further illustrate the invention. These Examples refer to bchla. The remaining compounds of formulae (1) and (2) have similar absorption spectra as they contain the same tetrahydroporphyrin resonance system, and have similar solubilities.

Example 1

Formulation of bchla

Bacteriochlorophyll-a, obtained at >90% purity from Porphyrin Products (Logan, UT) was dissolved at a concentration of 5 mg/ml in Tween-80 (Sigma) by stirring for several hours or overnight. The resulting solution was mixed with 9 volumes of Hank's buffer solution with agitation until all of the detergent solution was dissolved. Any remaining particulate matter is removed by filtration and the concentration of the final solution is determined spectrophotometrically using a 1:100 dilution in distilled water (OD_{780} = 87.3 for 1 mg/ml of concentrate). In general, if the initial solution of bchla is conducted carefully, the resulting formulation has a concentration of bchla of 0.5 mg/ml.

Example 2

Effect of bchla on Tumors

DBA2/HaD mice were transplanted with SMT-F tumors. When the subcutaneous tumors reached 4.5-5.5 mm in diameter, the mice, in groups of five, were injected intravenously with the bchla solution of Example 1 in doses of 5-30 mg/kg. At a time 1 hour-5 days later, the tumor, previously shaved and depiliated, plus a margin of 2-3 mm was exposed to radiation of a wavelength in the range 630-800 nm using a Spectraphysics argon dye laser with Exciton LDS751 dye, tunable over the 700-800 nm range or a diode laser--e.g., Spectra Diode emitting in the 750-850 nm range or a Xenon arc lamp filtered with an interference filter to pass 90% of the 700 nm light ±60 nm at dose rates of 75-150 mW/cm^2. When the Xenon system was used, mild hyperthermia resulted (42°C at 160 mW/cm^2). It is not known whether this temperature rise acts synergistically with bchla as has been shown with HpD and its active fraction.

Tumor response is shown in the table of Figure 1 for the seventh day after light treatment which indicates regression, and at a time point at least 30 days after light treatment, which would indicate cure, if there had been no regrowth.

As shown in Figure 1 good response to bchla was obtained, for example, after 2 hours at 5 mg/kg in the 670-790 nm range and after 24 hours after injection with 10 mg/kg and irradiated at 680-780 nm.

Figure 2 shows the action spectrum along with the absorption spectra of bchla, pheophytin (demetalated bchla, found in vivo) and for chlorophyll (oxidized bchla, theoretically found in vivo). The "X"s represent the 7 day response when 270 J/cm^2 were used 2 hours after the administration of 5 mg/kg; the squares represent the 7 day response when 270 J/cm^2 were administered 24 hours after administration of 10 mg/kg, and the circles represent the 30 day (cure) response, all as a function of wavelength of light used to treat the tumor.

Example 3

Determination of Therapeutic Ratio

One of the undesirable side effects of photodynamic therapy using certain compounds is cutaneous photosensitivity unrelated to the target biological substrate. Accordingly, the effect of the treatment on the photosensitivity of the foot of the treated mice was measured. The response of the foot was measured as erythema and/or edema (or loss of skin or further damage).

The results are shown in Figure 3. The left ordinate shows the percentage of tumors which responded; the right ordinate is an arbitrary scale for the foot response wherein 1.0 represents severe erythema and edema; 0.1 represents little effect, and 0.5 represents a moderate reaction. The results show that for bchla, the sensitivity of the tumor and the skin of the foot declined concomitantly, while for the active component of hematoporphyrin derivative designated DHE, the sensitivity persists for more than 10 days after injection. Thus, with DHE the tissue (foot) would be sensitive to light (for example, sunlight) for an extended period of time (30 days in humans), whereas for bchla, sensitivity could be expected to persist for only a few days.

Example 4

Metabolism of bchla

Uptake and clearance of bchla in tumor and liver were measured by extraction of the tumor or liver tissue with 1:1MeOH:CH_2Cl_2, followed by HPLC analysis. The levels of bchla in tumor and liver after injection of bchla are shown in Table 1.

Table 1

bchla Uptake in DBA/2 Ha Mice in SMT-F Tumor			
Dose bchla (mg/kg)	Time After Injection	Tissue Level (ug/g)	
		Tumor	Liver
10	2 h	6.14	44
10	24 h	-	49.4*
20	2 h	16	-
20	24 h	10-19.7*	-
10	48 h	10.7*	-

*Values at time intervals of 1 day or more are uncertain since preliminary experiments indicate conversion to other components (see below).

These results show that both tumor and liver have high levels of the compound after 2 hours and that these levels are maintained for as long as 24 or 48 hours.

However, partial conversion to bacteriopheophytin occurs at 24 hours or more in tumor and 2 hours in liver. Two hours after injection, the tumor contains essentially only bchla with a small amount of material wherein the phytyl group has hydrolyzed; at 48 hours the tumor contains mainly material without phytyl and without Mg. At 24 hours the material in tumor is demetallized but still contains phytyl.

Example 5

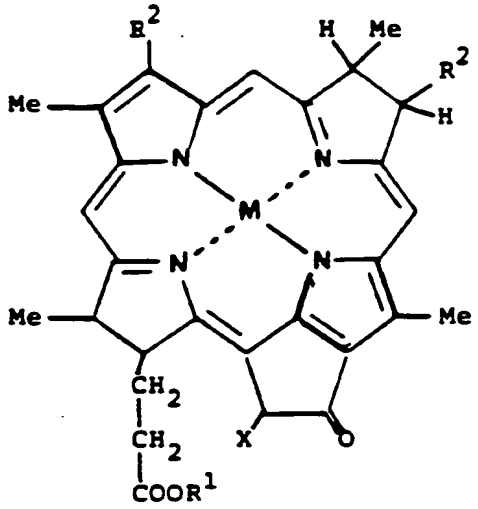
Light Penetration

Comparison was made using bchla at 20 mg/kg with irradiation after 1 hour at 270 J/cm^2 at 780 nm, and DHE at 5 mg/kg after 1 hour at 270 J/cm^2 at 630 nm. Animals with tumors approximately 1 cm in depth were used in the comparison. Histological sections were obtained the day following treatment, fixed and stained. A comparison using a total of 4 animals showed a necrotic depth of 5-6 mm for DHE and approximately 9 mm for bchla, consistent with deeper penetration of 780 nm light compared to 630 nm light.

Claims

1. An ex vivo method to effect the destruction or impairment of undesired target biological substrates in a biological fluid which method comprises:

   a) treating said biological substrates with a compound of formula (1) or (2):



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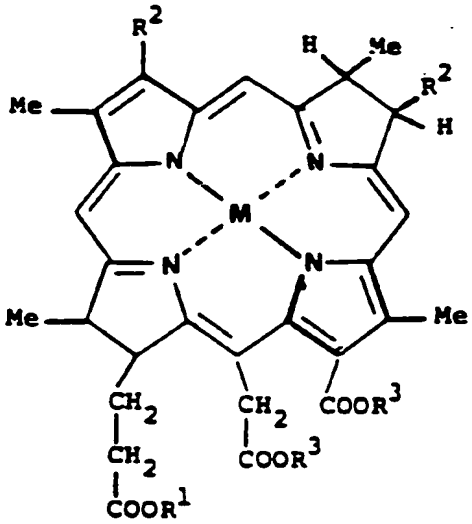


(2)

wherein M is a nonparamagnetic metal selected from Mg^{2+}, Sn^{2+}, and Zn^{2+}, or represents 2 H^+, each H^+ bonded to one of the N atoms connected by the solid lines;

R^1 is a saturated or unsaturated hydrocarbyl residue of 8-25 carbon atoms;

each R^2 is independently selected from vinyl, ethyl, acetyl and 1-hydroxyethyl; and

X is $COOR^3$, wherein R^3 is C_{1-4} alkyl;

in an amount of 1 to 100 µg/ml fluid to photosensitize said biological substrates to the resultant of irradiation absorbed by the compound of formula (1) or (2); and

(b) irradiating the treated biological substrates with radiation having a wavelength absorbed by the compound of formula (1) or (2).

2. The method of claim 1 wherein R^1 is a phytyl residue and M is Mg^{2+}

3. The method of claim 1 or 2 wherein one R^2 is acetyl and the other R^2 is vinyl or ethyl.

4. The method of claim 1 wherein the compound of formula (1) is bacteriochlorophyll-a or bacteriochlorophyll-b.

5. The method of any one of the preceding claims wherein the biological fluid is blood or blood plasma.

6. The method of any one of the preceding claims wherein the target biological substrate is selected from tumor cells, bacterial cells, fungi, protozoa and viruses.

7. The method of any one of the preceding claims wherein the radiation is generated by a diode laser.

8. Use of a compound of formula (1) or (2) as defined in any one of claims 1 to 4 in the manufacture of a composition for use in a method to effect the destruction or impairment of an undesired biological substrate or to locate a tumor in a subject, which method comprises:

administering said composition to said subject in an amount of 1 to 20 mg/kg body weight of the compound of formula (1) or (2); and
irradiating said undesired biological substrate or said tumor in vivo with radiation having a wavelength absorbed by the compound of formula (1) or (2).

9. Use according to claim 8 wherein said compound is administered in an amount of 1 to 3 mg/kg body weight.

10. Use according to claim 8 or 9 wherein the undesired biological substrate is a tumor.

11. Use of a compound of formula (1) or (2) as defined in any one of claims 1 to 4 in the manufacture of a composition for use in a method to effect the destruction or impairment of a pathogen, which comprises:

administering said composition to a subject infected with said pathogen in an amount of 1 to 20 mg/kg body weight of the compound of formula (1) or (2); and
irradiating tissue or fluid containing said pathogen in vivo or in vitro with radiation having a wavelength absorbed by the compound of formula (1) or (2).

12. Use according to claim 11 wherein said composition is administered in an amount of 1 to 3 mg/kg body weight of the compound of formula (1) or (2).

13. Use of a compound of formula (1) or (2) as defined in any one of claims 1 to 4 in the manufacture of a composition for use in a method of treatment of a skin disease, which comprises:

topically applying said composition to a subject with said skin disease in an amount of 1 to 20 mg/kg body weight of the compound of formula (1) or (2); and
irradiating the infectious virus or cells carrying the disease with radiation having a wavelength absorbed by the compound of formula (1) or (2).

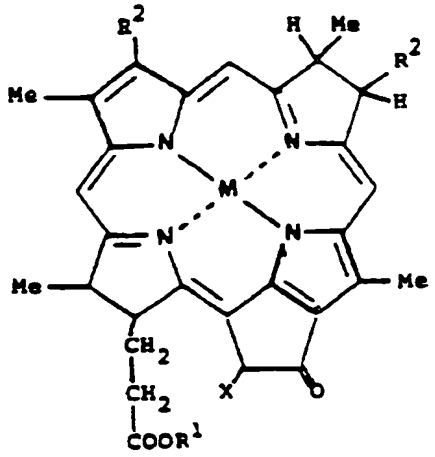
14. Use according to claim 13 wherein said composition is administered in an amount of 1 to 3 mg/kg body weight of the compound of formula (1) or (2).

15. A composition suitable for use in a method to effect the destruction or impairement of undesired target biological substrates by photodynamic treatment which comprises a compound of formula (1) or (2) as defined in any one of claims 1 to 4 in admixture with at least one pharmaceutically acceptable excipient which contains a liposome carrier.

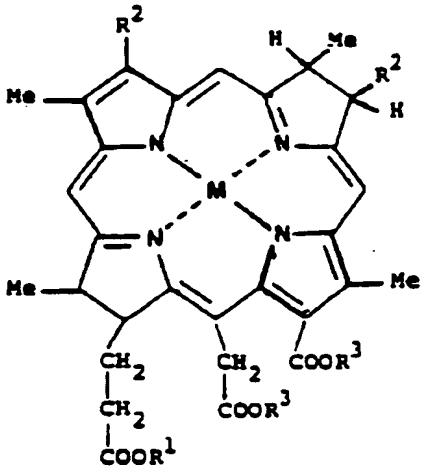
Patentansprüche

1. *ex-vivo*-Verfahren, um ein Zerstörung oder eine Beeinträchtigung von unerwünschten biologischen Zielsubstraten in einer biologischen Flüssigkeit zu bewirken, wobei das Verfahren folgendes umfaßt:

(a) Behandeln der biologischen Substrate mit einer Verbindung der Formel (1) oder (2):



(1)



(2)

worin M ein nicht-paramagnetisches aus Mg^{2+}, Sn^{2+} und Zn^{2+} gewähltes Metall oder 2 H^+ bedeutet, wobei jedes H^+ an einem der durch die durchgezogenen Linien verbundenen N-Atome gebunden ist;
R^1 ein gesättigter oder ungesättigter Kohlenwasserstoffrest mit 8-25 Kohlenstoffatomen ist;
jedes R^2 unabhängig gewählt wird aus Vinyl, Ethyl, Acetyl und 1-Hydroxyethyl; und
X $COOR^3$ ist, wobei R^3 C_{1-4}-Alkyl ist;
in einer Menge von 1 bis 100 µg/ml Flüssigkeit, um die biologischen Substrate gegenüber der durch die Verbindung der Formel (1) oder (2) absorbierten Strahlung photozusensibilisieren; und

(b) Bestrahlen der behandelten biologischen Substrate mit Strahlung einer durch die Verbindung der Formel (1) oder (2) absorbierten Wellenlänge.

2. Verfahren nach Anspruch 1, worin R^1 ein Phytylrest und M Mg^{2+} sind.

3. Verfahren nach Anspruch 1 oder 2, worin ein R^2 Acetyl ist und das anderer R^2 Vinyl oder Ethyl ist.

4. Verfahren nach Anspruch 1, worin die Verbindung der Formel (1) Bakteriochlorophyll-a oder Bakteriochlorophyll-b

ist.

5. Verfahren nach einem der vorhergehenden Ansprüche, bei dem die biologische Flüssigkeit Blut oder Blutplasma ist.

6. Verfahren nach einem der vorhergehenden Ansprüche, bei dem das biologische Zielsubstrat aus Tumorzellen, Bakterienzellen, Pilzen, Protozoen und Viren gewählt wird.

7. Verfahren nach einem der vorhergehenden Ansprüche, bei dem die Strahlung durch einen Diodenlaser erzeugt wird.

8. Verwendung einer Verbindung der Formel (1) oder (2), wie nach einem beliebigen der Ansprüch 1 bis 4 definiert, bei der Herstellung einer Zusammensetzung zur Verwendung bei einem Verfahren, um eine Zerstörung oder eine Beeinträchtigung eines unerwünschten biologischen Zielsubstrates zu bewirken oder einen Tumor in einem Subjekt zu lokalisieren, wobei das Verfahren folgendes umfaßt:

   Verabreichen der Zusammensetzung an das Subjekt in einer Menge von 1 bis 20 mg/kg Körpergewicht der Verbindung der Formel (1) oder (2); und

   Bestrahlen des ungewünschten biologischen Substrates oder des Tumors *in vivo* mit Strahlung einer Wellenlänge, die durch die Verbindung der Formel (1) oder (2) absorbiert wird.

9. Verwendung nach Anspruch 8, wobei die Verbindung in einer Menge von 1 bis 3 mg/kg Körpergewicht verabreicht wird.

10. Verwendung nach Anspruch 8 oder 9, wobei das unerwünscht biologische Substrat ein Tumor ist.

11. Verwendung einer Verbindung der Formel (1) oder (2), wie sie nach einem beliebigen der Ansprüche 1 bis 4 definiert ist, bei der Herstellung einer Zusammensetzung zur Verwendung bei einem Verfahren, um ein Pathogen zu zerstören oder zu beeinträchtigen, welches folgendes umfaßt:

   Verabreichung der Zusammensetzung an ein Subjekt, welches mit dem Pathogen infiziert ist, und zwar in einer Menge von 1 bis 20 mg/kg Körpergewicht der Verbindung der Formel (1) oder (2); und

   Bestrahlen des Gewebes oder der Flüssigkeit, die das Pathogen enthält, *in vivo* oder *in vitro* mit Strahlung einer Wellenlänge, die durch die Verbindung der Formel (1) oder (2) absorbiert wird.

12. Verwendung nach Anspruch 11, wobei die Zusammensetzung in einer Menge von 1 bis 3 mg/kg Körpergewicht der Verbindung der Formel (1) oder (2) verabreicht wird.

13. Verwendung der Verbindung der Formel (1) oder (2), wie sie nach einem beliebigen der Ansprüche 1 bis 4 definiert ist, bei der Herstellung einer Zusammensetzung zur Verwendung bei einem Verfahren der Behandlung einer Hautkrankheit, folgendes umfassend:

   Topisches Auftragen der Zusammensetzung bei einem Subjekt mit der Hautkrankheit in einer Menge von 1 bis 20 mg/kg Körpergewicht der Verbindung der Formel (1) oder (2); und
   Bestrahlen des infektiösen Virus oder der diese Krankheit tragenden Zellen mit Strahlung einer Wellenlänge, die durch die Verbindung der Formel (1) oder (2) absorbiert wird.

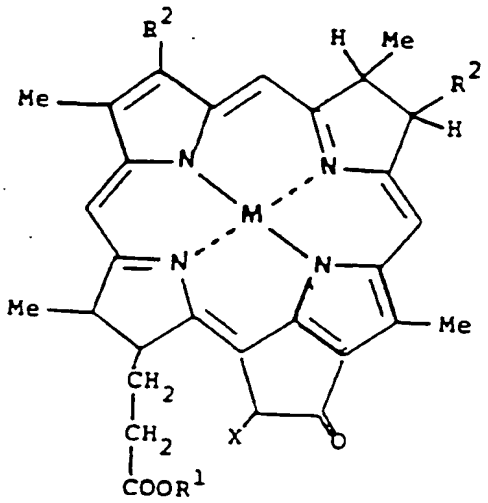
14. Verwendung nach Anspruch 13, wobei die Zusammensetzung in einer Menge von 1 bis 3 mg/kg Körpergewicht der Verbindung der Formel (1) oder (2) verabreicht wird.

15. Zusammensetzung, die zur Verwendung bei einem Verfahren geeignet ist, bei dem eine Zerstörung oder eine Beeinträchtigung von unerwünschten biologischen Zielsubstraten durch photodynamische Behandlung bewirkt wird, welche eine Verbindung der Formel (1) oder (2), wie sie nach einem beliebigen der Ansprüche 1 bis 4 definiert ist, in Vermischung mit mindestens einem pharmazeutisch annehmbaren Corrigens, welches einen Liposomträger enthält, umfaßt.

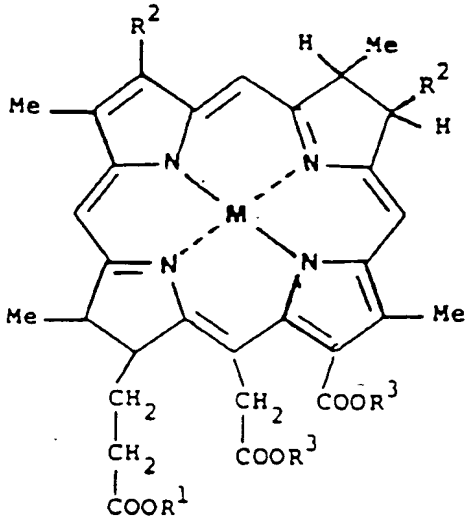
Revendications

1. Méthode ex vivo pour effectuer la destruction ou l'altération de substrats biologiques cibles indésirables dans un fluide biologique, méthode qui comprend les étapes consistant à :

a) traiter lesdits substrats biologiques avec un composé de formule (1) ou (2) :



(1)



(2)

dans lesquelles M est un métal non paramagnétique choisi parmi Mg^{2+}, Sn^{2+}, et Zn^{2+}, ou représente 2 H^+, chaque H^+ étant lié à un des atomes N relié par les lignes continues ;
R^1 est un résidu hydrocarbyle saturé ou insaturé de 8-25 atomes de carbone ;
chaque R^2 est choisi indépendamment parmi le groupe vinyle, éthyle, acétyle et 1-hydroxyéthyle ; et
X est $COOR^3$, où R^3 est un groupe alkyle en C_{1-4} :

en une quantité de 1 à 100 µg/ml de fluide, pour photosensibiliser lesdits substrats biologiques à l'effet d'une irradiation absorbée par le composé de formule (1) ou (2) ; et

(b) irradier les substrats biologiques traités avec une radiation ayant une longueur d'onde absorbée par le composé de formule (1) ou (2).

2. Méthode selon la revendication 1 dans laquelle R^1 est un résidu phytyle et M est Mg^{2+}.

3. Méthode selon la revendication 1 ou 2 dans laquelle un R^2 est un groupe acétyle et l'autre R^2 est un groupe vinyle ou éthyle.

4. Méthode selon la revendication 1 dans laquelle le composé de formule (1) est la bactériochlorophylle-a ou la bactériochlorophylle-b.

5. Méthode selon l'une quelconque des revendications précédentes dans laquelle le fluide biologique est du sang ou du plasma sanguin.

6. Méthode selon l'une quelconque des revendications précédentes dans laquelle le substrat biologique cible est choisi parmi des cellules tumorales, des cellules bactériennes, des champignons, des protozoaires et des virus.

7. Méthode selon l'une quelconque des revendications précédentes dans laquelle la radiation est générée par un laser à diode.

8. Utilisation d'un composé de formule (1) ou (2) tel que défini dans l'une quelconque des revendications 1 à 4 dans la préparation d'une composition utilisable dans une méthode pour effectuer la destruction ou l'altération d'un substrat biologique indésirable ou pour localiser une tumeur chez un sujet, méthode qui comprend les étapes consistant à :

administrer ladite composition audit sujet en une quantité de 1 à 20 mg/kg de poids corporel du composé de formule (1) ou (2) ; et

irradier ledit substrat biologique indésirable ou ladite tumeur in vivo avec une radiation ayant une longueur d'onde absorbée par le composé de formule (1) ou (2).

9. Utilisation selon la revendication 8 dans laquelle ledit composé est administré en une quantité de 1 à 3 mg/kg de poids corporel.

10. Utilisation selon la revendication 8 ou 9 dans laquelle le substrat biologique indésirable est une tumeur.

11. Utilisation d'un composé de formule (1) ou (2) tel que défini dans l'une quelconque des revendications 1 à 4 dans la préparation d'une composition utilisable dans une méthode pour effectuer la destruction ou l'altération d'un agent pathogène, qui comprend les étapes consistant à:

administrer ladite composition à un sujet infecté par ledit agent pathogène en une quantité de 1 à 20 mg/kg de poids corporel du composé de formule (1) ou (2) ; et

irradier le tissu ou le fluide contenant ledit agent pathogène in vivo ou in vitro avec une radiation ayant une longueur d'onde absorbée par le composé de formule (1) ou (2).

12. Utilisation selon la revendication 11 dans laquelle ladite composition est administrée en une quantité de 1 à 3 mg/kg de poids corporel du composé de formule (1) ou (2).

13. Utilisation d'un composé de formule (1) ou (2) tel que défini dans l'une quelconque des revendications 1 à 4 dans la préparation d'une composition utilisable dans une méthode de traitement d'une maladie de la peau, qui comprend les étapes consistant à :

effectuer une application topique de ladite composition à un sujet ayant ladite maladie de la peau en une quantité de 1 à 20 mg/kg de poids corporel du composé de formule (1) ou (2) ; et

irradier les virus infectieux ou cellules portant la maladie avec une radiation ayant une longueur d'onde absorbée par le composé de formule (1) ou (2).

14. Utilisation selon la revendication 13 dans laquelle la composition est administrée en une quantité de 1 à 3 mg/kg de poids corporel du composé de formule (1) ou (2).

15. Composition convenant pour une utilisation dans une méthode pour effectuer la destruction ou l'altération de substrats biologiques cibles indésirables par un traitement photodynamique qui comprend un composé de formule (1) ou (2) tel que défini dans l'une quelconque des revendications 1 à 4 en mélange avec au moins un excipient pharmaceutiquement acceptable qui contient un véhicule de type liposome.

Tumor Response in the SMT-F Tumor (DBA/2 Ha Mice)

Drug Dose g/kg, i.v.	Time Interval (h)	Wavelength (nm)	Light Dose (J/cm^2)	Tumor Response Day 7	Day 30+	
3.0	2	680	270	3/5	0	
"	2	750	270	3/6	0	
"	2	780	270	3/6	0	
5.0	2	780	270	18/26 (69%)	4/26 (15%)	
"	2	630	270	2/6	0	
"	2	670	270	6/6	0	
"	2	726	270	5/5	0	
"	2	740	270	3/3	0	
"	2	760	270	4/4	3/4	
"	2	790	270	3/6	1/6	
"	2	800	270	2/5	0	
10.0	24	630	270	0/5	0	
"	24	680	270	5/5	0	
"	24	750	270	9/10	0	
"	24	780	270	9/12	0	
"	24	799	270	0	0	
5.0	1	780	540	3/5	0	
10	1	780	540	5/5	4/5	
10	2	780	540	4/4	0	
20	2	780	540	5/5	1/5	
10	24	780	540	4/30	0	uncertain dosimetry
20	24	780	540	4/9	0	
30	24	780	540	4/5	0	
10	2	780	1080	2/5	1/5	
10	24	780	1080	10/13	0	
20	24	780	1080	4/5	1/5	
20	48	780	1080	5/5	0	
20	72	780	1080	0/7	0	
20	96	780	1080	2/5	0	
20	120	780	1080	0/5	0	
20	1	780	1080	3/3	1/3	
20	2	780	1080	4/4	2/4	

Note:

1. Data from groups containing fewer than 10 mice are not statistically significant and should be considered preliminary.

2. Curability (i.e. >30 day response) in this tumor system likely depends upon damage to normal vasculature adjacent to the tumor and may not be relevant to humans.

FIG. 1

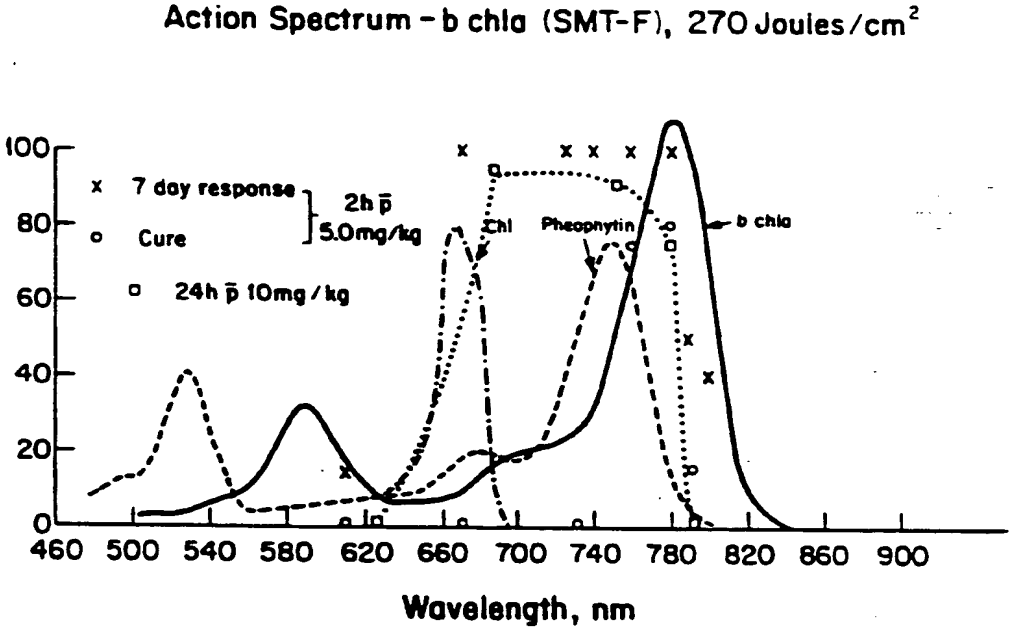

Action Spectrum - b chla (SMT-F), 270 Joules/cm²
100
80
60
40
20
0
x 7 day response
o Cure
2h p̄ 5.0mg/kg
24h p̄ 10mg/kg
Chl
Pheophytin
b chla
460 500 540 580 620 660 700 740 780 820 860 900
Wavelength, nm

FIG. 2

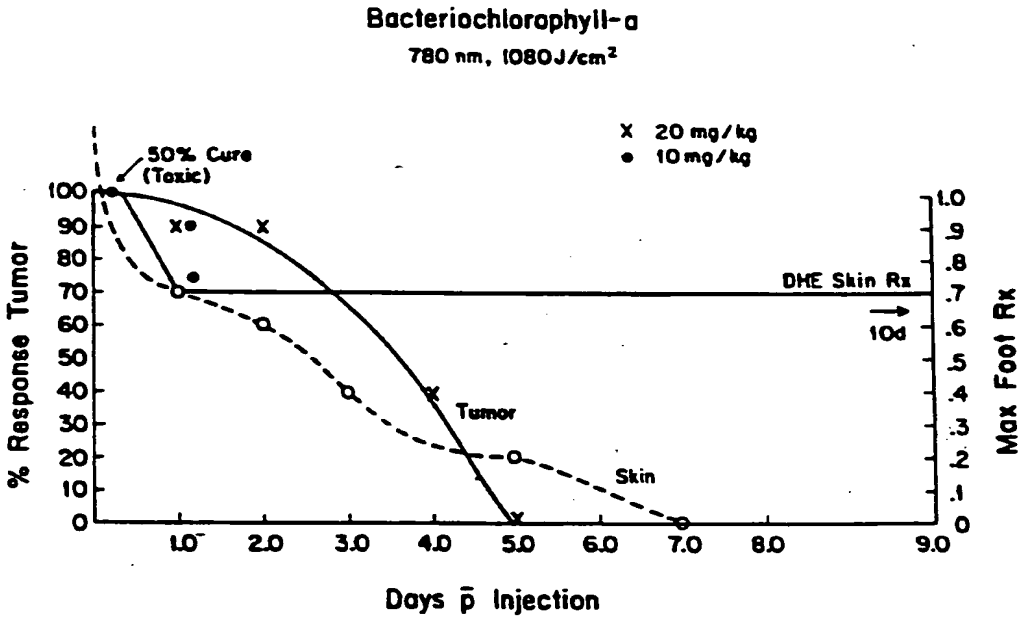

Bacteriochlorophyll-a
780 nm, 1080J/cm²
X 20 mg/kg
• 10 mg/kg
50% Cure
(Toxic)
DHE Skin Rx
10d
Tumor
Skin
% Response Tumor
Max Foot Rx
Days p̄ Injection
100
90
80
70
60
50
40
30
20
10
0
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0

FIG. 3